Construction of a Prognostic Signature in Ewing's Sarcoma: Based on RNA-binding Genes

Bo Wu

Jilin University

Haoqun Xie

Jilin University

Wang Yibo

Jilin University

Guo Zhen

Jilin University

Hou Yuanyuan

Jilin University

Dong Zhu (✉ zhu_dong@mails.jlu.edu.cn)

Jilin University First Hospital <https://orcid.org/0000-0002-0637-5757>

Research Article

Keywords: RNA-binding proteins, Ewing's sarcoma, prognosis, bioinformatics

Posted Date: May 11th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-482153/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: Ewing's sarcoma is the second most prevalent primary malignant bone neoplasm. RNA-binding proteins (RBPs) play a crucial role in post-transcriptional events. In tumor cells, the alterations of post-transcription enable cells to adapt to adjacent environment rapidly. Thus, the functions of RBPs in Ewing's sarcoma can be of high value in the prognostic [1]. The underlying mechanism between Ewing's sarcoma and RBPs remained unclear.

Methods: Based on the GEO dataset, we investigated the global protein expression profile of Ewing's sarcoma patients. Differentially expressed proteins and survival-related RNA-binding protein related genes (RRGs) were evaluated by computational difference algorithm and COX regression analysis. In addition, we also explored the mutations in these RRGs. A new prognostic indicator based on RRGs was developed and tested afterwards using multivariate COX analysis.

Results: The results showed that a total of 16 RRGs which closely associated with the overall survival in Ewing's sarcoma patients using multivariate Cox regression analysis. The prognosis-related RRGs signature established using Cox regression model consists of 8 RRGs that can divide patients into high-risk and low-risk groups. Our results suggested that overall survival rate of high-risk group patients was shorter than the patients in low-risk group. According to multivariate Cox analysis, risk score index was an independent prognosis factor for Ewing's sarcoma. In addition, the area under the curve of the corresponding receiver operating characteristic (ROC) curve of survival is 0.947.

Conclusion: The 8 RRGs marker can predict the prognosis of Ewing's sarcoma and thus help individualized treatment of patients at different risks.

Introduction

Ewing's sarcoma is the second most prevalent primary malignant bone neoplasm accounting for approximately 25–34% of malignant bone tumors, which is often occurring in children and young adults with male predominance [2, 3]. The tumors are mainly found in bones and adjacent soft tissue characterized by an aggressive small round blue cell malignant neoplasm [4, 5]. Symptoms of Ewing's sarcoma include localized pain, a palpable mass and local swelling and back pain. Patients may also present fever, fatigue, weight loss, discomfort and lack of appetite in cases involving metastases [6]. Multiple studies have proven that tumor-specific fusion protein can be encoded by the Ewing sarcoma breakpoint region (EWSR) gene on chromosome 22 and a member of the ETS family of transcription factors implicated in the Ewing sarcoma pathogenetic process which are induced by chromosomal translocation [7–9]. Currently, the principal strategies for treatments of Ewing's sarcoma, including surgical excision, local radiotherapy and multidrug chemotherapy, are still far from satisfactory despite the enormous progress achieved along with technological and science advancements [10]. Statistics suggested that the five-year survival rate for Ewing's sarcoma patients is only 50%, for those who are presented with metastases, their five-year survival rate dropped to 30% [5, 11]. The underlying mechanisms of Ewing's sarcoma cannot be fully illustrated because of its complexity. The lack of predictive biomarkers may lead to inaccurate judgments on the patient's prognosis which causes patients' relapse or metastasis due to inadequate treatment. Thus, the valid biomarkers would contribute greatly to clinical treatment and prognostic [12].

RNA-binding proteins (RBP) regulate RNA processing at multiple levels, including mRNA stability, mRNA localization, translation efficiency and alternative splicing, playing a crucial role in post-transcriptional events. In tumor cells, the alterations of post-transcription enable cells to adapt to adjacent environment rapidly [1, 9]. The importance of RBPs have long been overlooked not only because of the sensitivity of gene expression detection systems was just advanced recently for the systematic study of RBPs, but also because of a study suggesting that the changes in RBPs mRNA expression levels are small [13]. Multiple studies have suggested that RBPs are involved in a wide range of biological processes, including apoptosis, tumorigenesis and reproductive development, small alterations in the expression hold an opportunity to lead to a large-scale disruption of downstream regulatory networks. Thus, RBPs are closely associated with multiple human diseases. The understanding of the differentially expressed RBPs will greatly contribute to the studying of pathogenesis of diseases to better identify the stage of tumor and screening of innovative drug targets [14].

We used to believe that the core of cancer lies in the gene mutations. But there are increasing number of research proving that the changes in the gene expression without accompanied gene mutations contribute to the tumor adjustment and development. These

changes are dominated by RBPs in the post-transcriptional events[15]. The expression of RBPs was found to be different in cancer cells compared with adjacent normal cells in multiple malignancies [16–22], of which the mechanisms are illustrated in the study[23], dysregulated RBPs influence the expression levels of target RNAs related to cancer phenotypes, such as proliferation, apoptosis, senescence, angiogenesis, and EMT/invasion/metastasis.

In this study, we identified the differentially expressed RNA-binding protein related genes(RRGs) in Ewing's sarcoma tissue, some of which might be used as potential prognostic biomarkers in the future.

Results

Identification of differentially expressed RRGs.

We downloaded the RNA-seq data of 64 Ewing's sarcoma tissue samples and 18 sarcoma-free samples from GEO. The expression values of 56 RNA-binding-protein-related genes (RRGs) were extracted. Taken the criteria of $FDR < 0.05$ and $[\log_2(\text{fold change})] > 0.5$ into consideration, we finally obtained 15 up-regulated and 41 down-regulated RRGs (Figure 1A and 1B).

Also, the illustration of those differentially expressed RRGs between Ewing's sarcoma and tumor-free tissue is presented in the scatter plot (Figure 1C).

Functional enrichment of the differentially expressed RRGs

Functional enrichment analysis of 56 differentially expressed RRGs provides a biological understanding of these genes. GO bubble and GO circle (Figure 2A and 2B) summarized the GO enrichment of differentially expressed genes. Figure 2C is colored based on its cluster ID, and Figure 2D is colored by its p-value. Figure 2E shows that these RRGs were significantly enriched in mRNA processing, metabolism of RNA, translation, mRNA catabolic process and so on.

Identification of prognostic RRGs

We screened for RRGs which were greatly associated with prognosis to analyze RRGs which is involved in Ewing's sarcoma progression and 16 of them were chosen. The forest map of the hazard ratio illustrated that most of these genes are protective factors (Figure 3A).

Based on Cox regression analysis, we constructed RNA-binding proteins prognostic index (RPI) to divide Ewing's sarcoma patients into high risk and low risk groups.

The heatmap presented in Figure 3B shows the expression profile of the included RRGs.

According to the corplot, gene ZC3HAV1, PAN3, PAPD4, SRSF7, INTS6, PRPF38B, SRSF11, SRSF3, CAPRIN1, STRBP, MYEF2 and NXT2 are positively correlated with EEF1A2, RBM24, APOBEC2 and CPEB3. The rest of their correlations are negative (Figure 3C). Red is positively correlated and blue is negatively correlated. The area of the color on the upper right and the shade of the color on the lower left represent the correlation respectively. The larger the area and the darker the color is, the greater the correlation is.

To determine the performance of the RPI in predicting clinical outcomes among Ewing's sarcoma patients, the survival curves were also presented to analyze different survival times between high-risk and low-risk groups. Figure 3D indicated that patients in the high-risk group have a shorter survival time. The area under the curve of the corresponding receiver operating characteristic (ROC) curve is 0.947. This indicated that the prognostic index based on RRGs has a certain potential in survival prediction (Figure 3E).

Clinical utility of prognostic signature

Univariate analysis of risk score index showed that risk score index was associated with Ewing's sarcoma prognosis ($P < 0.05$, hazard ratio =1.087, 95% confidence interval =1.058-1.116)(Figure 4A). In addition, after adjusting for gender, age, and PRS type

showed that risk score index was an independent predictor of Ewing's sarcoma prognosis in multivariate Cox regression analysis ($P < 0.05$, hazard ratio = 1.094, 95% confidence interval = 1.064-1.125) (Figure 4B). Multivariate analysis suggested that RPI was significantly associated with patient prognosis.

Through the analysis of the differentially expressed genes in the high and low risk groups, it can be seen that among the 6 genes (Caprin1, PAN3, EEF1A2, SRSF11, ZC3HAV1, SRSF7) that are significantly related to prognosis, except for gene EEF1A2, the expression in the high risk group is significantly higher than that in the low risk group. (Figure 4C)

Construction of the nomogram model.

A Nomogram model was established based on multivariate regression analysis to roughly calculate the survival rate of this Ewing's sarcoma patients at one, three, and five years. (Figure 4D). Figure 4E compares the observed value to the Nomogram prediction. The graph shows that the observed value and predicted value can reach the corresponding relationship, which also proves the correctness of this detection method to a certain extent. 1-year, 3-year and 5-year AUC are 0.870, 0.912 and 0.865 respectively, which means the authenticity of our test was pretty good.

Immune cell enrichment analysis

To better understand the characteristics of immune cells and their relations with RRGs, the TIMER database was used to analyze the correlation between the abundance of immune cells and the ten prognostic genes (APOBEC2, CAPRIN1, CPEB3, EEF1A2, INTS6, MYEF2, NXT2, PAN3, PAPD4 and PRPF38B). The results revealed that gene CAPRIN1 is positively associated with B cell ($P=0.036$) and negatively associated with CD4+ T cell ($P<0.001$). CPEB3 has negative correlations with myeloid dendritic cell ($P<0.001$) and positive correlations with B cell neutrophil, CD4+ T cell ($P=0.006$, 0.042 respectively). EEF1A2 is negatively correlated with myeloid dendritic cell and neutrophil ($P<0.001$, 0.001 respectively) and positively associated with B cell ($P=0.030$). INTS6 has positive correlations with B cell and CD8+ T cell ($P=0.005$, 0.018 respectively) and negative correlations with macrophage, myeloid dendritic cell and CD4+ T cell ($P<0.001$, 0.001 0.001 respectively). MYEF2 is positively associated with B cell ($P=0.003$) and negatively associated with macrophage, myeloid dendritic cell, neutrophil and CD8+ T cell ($P<0.001$, 0.001 , $=0.001$, $=0.002$ respectively). NXT2 is positively related with B cell, neutrophil and CD8+ T cell ($P=0.003$, 0.005 , 0.047 respectively) and negatively related with CD4+ T cell ($P=0.010$). PAN3 has positive correlations with B cell ($P=0.005$) and negative correlations with myeloid dendritic cell ($P<0.001$). PAPD4 is positively related with neutrophil ($P=0.024$). PRPF38B is positively associated with B cell neutrophil and CD8+ T cell ($P<0.001$, $=0.005$, $=0.034$ respectively). The details are shown in Figure 5-6.

Discussion

Although the significance of the RNA-binding protein in the prognosis of Ewing's sarcoma has been demonstrated in numerous studies, its clinical significance has not been demonstrated by a comprehensive analysis of RRGs. In order to analyze the Ewing's sarcoma related genes from the perspective of RNA-binding-protein, we screened and identified 16 prognostic RRGs. A prognostic model based on 16 RRGs which can be used for prognostic stratification in Ewing's sarcoma patients is constructed by our results, contributing to develop individualized treatment options based on patients' risk.

Metascape shows that the RRGs were significantly enriched in mRNA processing, metabolism of RNA, translation, mRNA catabolic process and so on, which are essential processes that dictate many aspects of cellular survival, proliferation, and differentiation. The processing of mRNA is closely related to the occurrence and development of cancer. Cancer acquires its specificity and produces different types or quantities of proteins through a series of mRNA processing processes, such as specific excision of introns and splicing of exons in mRNA [24]. RNA degradation is the crux of post-transcriptional regulation. RNA degradation event is closely related to RNA homeostasis, which can be affected by many factors such as the expression of DIS3 gene. Mutations in the RNA degradation-related regulatory gene DIS are involved in a large number of RNA degradation processes and are associated with the development and progression of several cancers, such as multiple myeloma [25]. Previous studies have shown that the occurrence of Ewing's sarcoma can be suppressed by inhibiting some genetic targets, thus inhibiting the corresponding precursor mRNA processing processes [26, 27]. The level of RNA in cells is highly regulated by RNA metabolism which is a very important

part of eukaryotic cell metabolism. According to a recent review, RNA metabolism is closely related to cell viability[28]. The precise metabolic process of RNA greatly affects the normal physiological activities of neurons and determines the types and quantities of proteins in neurons[29]. Cancerous mutations can occur in the process of translation, and the smooth translation of cancer-related genes is closely related to the occurrence of cancer. The imbalance in the translation process can affect the growth, reproduction and migration of cancer cells[30]. Translation inhibitors of mRNA may be used as targeted therapies for cancer[31].

Moreover, RNA splicing is a highly finetuned and intricate process that is susceptible to alterations during tumorigenesis. The emergence of somatic mutations in spliceosomal proteins or dysregulated expression of RBP splicing factors contribute to mis-spliced mRNA transcripts that support cancer growth[32]. The 16 RRGs can be of great significance to the progression of Ewing's sarcoma. According to the ROC curve, the 16 RRGs which were screened out can be of prognostic stratification in Ewing's sarcoma patients.

We identified a group of RRGs that predict the prognosis of Ewing's sarcoma patients. Most of these genes have been reported in previous studies to be significantly related to the prognosis of other malignancies. In the study[33],CAPRIN1 was proven to be positively related with circ-0000885 which was highly expressed in osteosarcoma cells. According to the study [34], the expression of PAN3 is lower among patients with chronic myeloid leukemia compared with healthy individuals. The down-regulation of CPEB3 promoted migration, proliferation and invasion in colorectal cancer cells and vice versa. The decreasing expression of CPEB3 is closely associated with poor prognosis in patients with colorectal cancer (47 vs. 62 months, $P = 0.035$, $n=99$)[35]. Gene EEF1A2 was proven to be over-expressed in multiple cancer tissue and has long been reputed as an oncogene.[36, 37] . SRSF3 has an oncogenic function, the study[38] illustrated the underlying mechanism. The SRSF3 splicer regulated the expression profile of the pyruvate kinase, which is one of the rate-limiting enzymes in glycolysis. Cancer cells mainly rely on a glycolysis-dominant energy metabolism. SRSF11, also known as SRP54(signal recognition protein 54), is a conserved component of the ribonucleoprotein complex that mediates cotranslational targeting and translocation of proteins to the endoplasmic reticulum. In the study[39],SRSF11 is identified as a novel TREC(telomerase RNA component) -binding protein which localizes to nuclear speckles. In this study, SRSF11 also associates with telomeres through an interaction withTRF2, which facilitates translocation of telomerase to telomeres. ZC3HAV1, which is also called Poly(ADP-ribose) Polymerase-13 (PARP13) or Zinc-finger Antiviral Protein (ZAP), modulates the miRNA silencing pathway to globally impact miRNA targets and regulates the translation of specific mRNAs. ZC3HAV1 helps to regulate the cellular response to the TNF-related apoptosis-inducing ligand and restrict the proliferation of oncogenic viruses.[40, 41] The underlying mechanism was illustrated in the study[42]. SRSF7 knockdown promotes apoptosis colon and lung cancer cells[43]. SRSF7 was of high expression in colon and lung cancer tissues, which also indicated poor prognosis[44, 45].

Limitations cannot be denied in this study. Firstly, we adopted a retrospective method of study, so the inherent bias cannot be avoided. Secondly, the prognostic model we constructed perhaps should be optimized by other independent cohorts. Thirdly, not all the underlying mechanisms of the prognostic genes are thoroughly illustrated in the studies we currently found, so further experiments will be needed in the future.

In conclusion, multiple Ewing's sarcoma prognostic RRGs are identified in this study based on corresponding clinical features and a comprehensive analysis of RRGs expression profiles. The genes identified in the RNA-binding protein regulation pathway also provide new possibilities for Ewing's sarcoma therapeutic intervention. A new risk scoring model was constructed based on the molecular features of RNA-binding protein related genes to effectively assess the prognosis of Ewing's sarcoma patients. However, further studies are needed to validate the findings of this study in order to aid clinical treatment.

Materials And Methods

RNA-binding protein related genes (RRGs)

We extracted a total of RRGs from the Geo database (<https://www.ncbi.nlm.nih.gov/geo/>), providing an up-to-date and complete human genes related to RNA-binding protein.

GEO data acquisition

64 Ewing's sarcoma patients were included. We got the expression levels of ferroptosis-related genes and clinical characteristics from the Gene Expression Omnibus (GEO) dataset (GSE17679)[46]. Survival-related RNA-binding protein related genes (RRGs) between tumor samples and non-tumor samples were identified according to the criteria: False discovery rate (FDR) <0.05 and $[\log_2(\text{fold change})] > 1$. Univariate Cox regression was adopted to identify patient survival associated RRGs for subsequent model construction.

Functional analysis

To perform functional enrichment of differentially expressed RRGs, we adopted the DAVID database Online Enrichment Tool (version 6.8, <https://david.ncifcrf.gov/tools.jsp/>). To assess relevant functional categories, Gene Ontology (GO) were adopted. GO enrichment pathways with p and q values less than 0.05 are considered to be significant categories. RRGs were also uploaded to online tools: Metascape, online websites with gene annotation, visualization and providing gene attributes ADDIN EN.CITE.

Construction of RRGs related prognostic model

Multivariate Cox regression was used to construct prognosis-related genes. A risk score formula for each patient was constructed and weighted by its estimated regression coefficients in a multivariate cox regression analysis after incorporating the expression values for each particular gene. Median risk score was used as the cut-off point based on the risk scoring formula, thus the patients were divided into low-risk and high-risk groups. Log-rank statistical methods and Kaplan-Meier were adopted respectively to compare and assess the survival differences between the two groups. To examine the role of risk scores in predicting patient outcomes, we used stratified analysis and multivariate cox regression analysis. ROC curves were adopted to study the accuracy of model predictions.

Statistical analysis

Kaplan-Meier method and log-rank test were adopted respectively to generate and compare the survival curves. We used the cox proportional hazard model to perform multivariate analysis. All statistical analyses were performed using the R language (version 3.6). All statistical tests were bilateral, with $p < 0.05$ being statistically significant.

Abbreviations

RNA-binding proteins, RBPs;

RNA-binding protein related genes, RRGs

the Cancer Genome Atlas, TCGA;

Differentially expressed genes, DEGs;

Receiver operating characteristic, ROC;

Gene ontology, GO;

Kyoto Encyclopedia of Genes and Genomes, KEGG;

Ewing's sarcoma breakpoint region, EWSR;

E-twenty six family, ETS family;

RNA-binding proteins prognostic index, RPI;

Zinc-Finger Antiviral Protein, ZAP;

Cell cycle associated protein 1, CAPRIN1;

Cytoplasmic polyadenylation element binding protein 3, CPEB3;

Poly(A) specific ribonuclease subunit, PAN3;

Serine And Arginine Rich Splicing Factor 3, SRSF3;

Serine And Arginine Rich Splicing Factor 11, SRSF11;

Serine And Arginine Rich Splicing Factor 11, SRSF7;

Eukaryotic Translation Elongation Factor 1 Alpha 2, EEF1A2.

Declarations

Conflict of interest

There is no conflict of interests.

Author contribution

WB designed the study and collected data. XHQ drafted the manuscript. WYB contributed to the writing. GZ and HYY provided critical feedback and contributed to the review of the manuscript. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Natural Science Foundation of China (Grant No12072129.).

References

1. Pereira, B., M. Billaud, and R. Almeida, *RNA-Binding Proteins in Cancer: Old Players and New Actors*. Trends Cancer, 2017. **3**(7): p. 506-528.
2. Huh, W.W., et al., *Ewing sarcoma family of tumors in children younger than 10 years of age*. Pediatr Blood Cancer, 2017. **64**(4).
3. Buckley, J.D., et al., *Epidemiology of osteosarcoma and Ewing's sarcoma in childhood: a study of 305 cases by the Children's Cancer Group*. Cancer, 1998. **83**(7): p. 1440-8.
4. McArdle, D.J.T., et al., *Extraosseous Ewing sarcoma arising in a chronically lymphedematous limb*. J Vasc Surg Cases Innov Tech, 2018. **4**(3): p. 210-215.
5. Grünwald, T.G.P., et al., *Ewing sarcoma*. Nat Rev Dis Primers, 2018. **4**(1): p. 5.
6. da Silva Ferreira, V., et al., *Cellular toxicology and mechanism of the response to silver-based nanoparticle exposure in Ewing's sarcoma cells*. Nanotechnology, 2021. **32**(11): p. 115101.
7. Delattre, O., et al., *Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours*. Nature, 1992. **359**(6391): p. 162-5.
8. Sankar, S. and S.L. Lessnick, *Promiscuous partnerships in Ewing's sarcoma*. Cancer Genet, 2011. **204**(7): p. 351-65.
9. Li, G., et al., *Identification of key genes and pathways in Ewing's sarcoma patients associated with metastasis and poor prognosis*. Onco Targets Ther, 2019. **12**: p. 4153-4165.
10. Ozaki, T., *Diagnosis and treatment of Ewing sarcoma of the bone: a review article*. J Orthop Sci, 2015. **20**(2): p. 250-63.
11. Damron, T.A., W.G. Ward, and A. Stewart, *Osteosarcoma, chondrosarcoma, and Ewing's sarcoma: National Cancer Data Base Report*. Clin Orthop Relat Res, 2007. **459**: p. 40-7.
12. Huang, L., et al., *A case report of misdiagnosis of Ewing's sarcoma of the duodenum*. Asian J Surg, 2021. **44**(1): p. 394-395.

13. Wang, J., Q. Liu, and Y. Shyr, *Dysregulated transcription across diverse cancer types reveals the importance of RNA-binding protein in carcinogenesis*. BMC Genomics, 2015. **16 Suppl 7**(Suppl 7): p. S5.
14. Wang, K., et al., *Integrated Bioinformatics Analysis the Function of RNA Binding Proteins (RBPs) and Their Prognostic Value in Breast Cancer*. Front Pharmacol, 2019. **10**: p. 140.
15. Wurth, L. and F. Gebauer, *RNA-binding proteins, multifaceted translational regulators in cancer*. Biochim Biophys Acta, 2015. **1849**(7): p. 881-6.
16. Truitt, M.L. and D. Ruggero, *New frontiers in translational control of the cancer genome*. Nat Rev Cancer, 2016. **16**(5): p. 288-304.
17. Hopkins, T.G., et al., *The RNA-binding protein LARP1 is a post-transcriptional regulator of survival and tumorigenesis in ovarian cancer*. Nucleic Acids Res, 2016. **44**(3): p. 1227-46.
18. Kobayashi, T., et al., *Decreased RNA-binding motif 5 expression is associated with tumor progression in gastric cancer*. Tumour Biol, 2017. **39**(3): p. 1010428317694547.
19. Wang, Q., et al., *RNA-binding protein RBM6 as a tumor suppressor gene represses the growth and progression in laryngocarcinoma*. Gene, 2019. **697**: p. 26-34.
20. García-Cárdenas, J.M., et al., *Post-transcriptional Regulation of Colorectal Cancer: A Focus on RNA-Binding Proteins*. Front Mol Biosci, 2019. **6**: p. 65.
21. Jain, A., et al., *Evaluation of Post-transcriptional Gene Regulation in Pancreatic Cancer Cells: Studying RNA Binding Proteins and Their mRNA Targets*. Methods Mol Biol, 2019. **1882**: p. 239-252.
22. Wurth, L., et al., *UNR/CSDE1 Drives a Post-transcriptional Program to Promote Melanoma Invasion and Metastasis*. Cancer Cell, 2016. **30**(5): p. 694-707.
23. Kang, D., Y. Lee, and J.S. Lee, *RNA-Binding Proteins in Cancer: Functional and Therapeutic Perspectives*. Cancers (Basel), 2020. **12**(9).
24. Desterro, J., P. Bak-Gordon, and M. Carmo-Fonseca, *Targeting mRNA processing as an anticancer strategy*. Nat Rev Drug Discov, 2020. **19**(2): p. 112-129.
25. Saramago, M., et al., *The Implication of mRNA Degradation Disorders on Human Disease: Focus on DIS3 and DIS3-Like Enzymes*. Adv Exp Med Biol, 2019. **1157**: p. 85-98.
26. Fidaleo, M., et al., *Genotoxic stress inhibits Ewing sarcoma cell growth by modulating alternative pre-mRNA processing of the RNA helicase DHX9*. Oncotarget, 2015. **6**(31): p. 31740-57.
27. Ross, N.T., et al., *CPSF3-dependent pre-mRNA processing as a druggable node in AML and Ewing's sarcoma*. Nat Chem Biol, 2020. **16**(1): p. 50-59.
28. Tatosyan, K.A., I.G. Ustyantsev, and D.A. Kramerov, *[RNA Degradation in Eukaryotic Cells]*. Mol Biol (Mosk), 2020. **54**(4): p. 542-561.
29. Weskamp, K. and S.J. Barmada, *RNA Degradation in Neurodegenerative Disease*. Adv Neurobiol, 2018. **20**: p. 103-142.
30. Sriram, A., J. Bohlen, and A.A. Teleman, *Translation acrobatics: how cancer cells exploit alternate modes of translational initiation*. EMBO Rep, 2018. **19**(10).
31. Pal, I., et al., *Targeting Translation of mRNA as a Therapeutic Strategy in Cancer*. Curr Hematol Malig Rep, 2019. **14**(4): p. 219-227.
32. Wang, E. and I. Aifantis, *RNA Splicing and Cancer*. Trends Cancer, 2020. **6**(8): p. 631-644.
33. Liu, X.Y., et al., *Caprin1 targeted by circular circ_0000885 in the tumor progression of osteosarcoma*. Eur Rev Med Pharmacol Sci, 2020. **24**(17): p. 8631.
34. Mascarenhas Cdo, C., et al., *Identification of target genes using gene expression profile of granulocytes from patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors*. Leuk Lymphoma, 2014. **55**(8): p. 1861-9.
35. Fang, Y., et al., *CPEB3 functions as a tumor suppressor in colorectal cancer via JAK/STAT signaling*. Aging (Albany NY), 2020. **12**(21): p. 21404-21422.
36. Zang, W., et al., *Retraction Note: miR-663 attenuates tumor growth and invasiveness by targeting eEF1A2 in pancreatic cancer*. Mol Cancer, 2019. **18**(1): p. 118.

37. Lee, M.H. and Y.J. Surh, *eEF1A2 as a putative oncogene*. Ann N Y Acad Sci, 2009. **1171**: p. 87-93.
38. Kuranaga, Y., et al., *SRSF3, a Splicer of the PKM Gene, Regulates Cell Growth and Maintenance of Cancer-Specific Energy Metabolism in Colon Cancer Cells*. Int J Mol Sci, 2018. **19**(10).
39. Lee, J.H., et al., *Involvement of SRSF11 in cell cycle-specific recruitment of telomerase to telomeres at nuclear speckles*. Nucleic Acids Res, 2015. **43**(17): p. 8435-51.
40. Gao, G., X. Guo, and S.P. Goff, *Inhibition of retroviral RNA production by ZAP, a CCCH-type zinc finger protein*. Science, 2002. **297**(5587): p. 1703-6.
41. Mao, R., et al., *Inhibition of hepatitis B virus replication by the host zinc finger antiviral protein*. PLoS Pathog, 2013. **9**(7): p. e1003494.
42. Todorova, T., F.J. Bock, and P. Chang, *Poly(ADP-ribose) polymerase-13 and RNA regulation in immunity and cancer*. Trends Mol Med, 2015. **21**(6): p. 373-84.
43. Fu, Y. and Y. Wang, *SRSF7 knockdown promotes apoptosis of colon and lung cancer cells*. Oncol Lett, 2018. **15**(4): p. 5545-5552.
44. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2015*. CA Cancer J Clin, 2015. **65**(1): p. 5-29.
45. Chen, W., et al., *Cancer statistics in China, 2015*. CA Cancer J Clin, 2016. **66**(2): p. 115-32.
46. Savola, S., et al., *High Expression of Complement Component 5 (C5) at Tumor Site Associates with Superior Survival in Ewing's Sarcoma Family of Tumour Patients*. ISRN Oncol, 2011. **2011**: p. 168712.

Tables

Table 1					
Descriptions of different Go IDs					
ID	Description	BgRatio	p.adjust	qvalue	Count
GO:0070482	response to oxygen levels	394/18670	4.56E-05	2.50E-05	8
GO:0006914	autophagy	496/18670	8.74E-05	4.80E-05	8
GO:0061919	process utilizing autophagic mechanism	496/18670	8.74E-05	4.80E-05	8
GO:0071496	cellular response to external stimulus	339/18670	8.74E-05	4.80E-05	7
GO:0043281	regulation of cysteine-type endopeptidase activity involved in apoptotic process	215/18670	0.000101	5.55E-05	6
GO:0036293	response to decreased oxygen levels	370/18670	0.000105	5.77E-05	7
GO:0071453	cellular response to oxygen levels	234/18670	0.000118	6.45E-05	6
GO:2000116	regulation of cysteine-type endopeptidase activity	239/18670	0.000118	6.45E-05	6
GO:0052548	regulation of endopeptidase activity	425/18670	0.000178	9.76E-05	7
GO:0052547	regulation of peptidase activity	452/18670	0.000242	0.000133	7
GO:0031667	response to nutrient levels	499/18670	0.000364	0.0002	7
GO:0000422	autophagy of mitochondrion	76/18670	0.000364	0.0002	4
GO:0061726	mitochondrion disassembly	76/18670	0.000364	0.0002	4
GO:0010506	regulation of autophagy	327/18670	0.000389	0.000214	6
GO:0071214	cellular response to abiotic stimulus	331/18670	0.000389	0.000214	6
hsa05163	Human cytomegalovirus infection	225/8041	8.23E-07	4.00E-07	9
hsa05131	Shigellosis	239/8041	0.000242	0.000118	7
hsa05219	Bladder cancer	41/8041	0.000253	0.000123	4
hsa05167	Kaposi sarcoma-associated herpesvirus infection	189/8041	0.000445	0.000216	6
hsa05205	Proteoglycans in cancer	205/8041	0.000567	0.000276	6
hsa04012	ErbB signaling pathway	85/8041	0.002088	0.001015	4
hsa05210	Colorectal cancer	86/8041	0.002088	0.001015	4
hsa04621	NOD-like receptor signaling pathway	181/8041	0.002614	0.001271	5
hsa04210	Apoptosis	136/8041	0.008178	0.003977	4
hsa04140	Autophagy - animal	137/8041	0.008178	0.003977	4
hsa05213	Endometrial cancer	58/8041	0.008178	0.003977	3
hsa04921	Oxytocin signaling pathway	154/8041	0.009795	0.004764	4
hsa05160	Hepatitis C	155/8041	0.009795	0.004764	4
hsa05230	Central carbon metabolism in cancer	69/8041	0.009795	0.004764	3

Table 2

Univariate and multivariate Cox regression analysis for ES prognosis

id	HR	HR.95L	HR.95H	pvalue	id	HR	HR.95L	HR.95H	pvalue
age	1.032559	1.015797	1.049598	0.000125	age	1.012377	0.994477	1.030598	0.176543
gender	0.882027	0.599785	1.297084	0.523485	gender	1.321754	0.853621	2.046615	0.211119
riskScore	2.446438	1.445822	4.139554	0.000857	riskScore	3.13598	1.726255	5.69694	0.000175
radiation	0.228438	0.145968	0.357503	1.04E-10	radiation	0.232215	0.109395	0.492929	0.000144
chemo	0.507945	0.343228	0.75171	0.000707	chemo	0.986192	0.515684	1.885989	0.966472
Subtype	1.0445	0.878744	1.241521	0.62143	Subtype	0.946025	0.776592	1.152425	0.581607
neoplasm type	0.689471	0.425558	1.117051	0.130956	neoplasm type	0.662314	0.391438	1.120635	0.124661

Figures

Figure 1

Heat map (A) and volcano map (B) show differentially expressed genes between Ewing's sarcoma and normal tissues, with red dots representing significantly up-regulated genes, green dots representing significantly down-regulated genes, and black dots representing no differences gene. (C) Expression patterns of 56 RNA-binding protein-related genes (RRGs) in Ewing's sarcoma types and paired non-tumor samples. Each red box plot represents a different tumor sample and green represents a non-tumor sample.

Figure 2

Gene functional enrichment of differentially expressed RRGs. (A-B) We adopted DAVID database Online Enrichment to perform GO analysis which shows the biological processes and molecular functions involved in differentially expressed RRGs. (C)colored by cluster ID, where nodes that share the same cluster ID are typically close to each other in RRGs. (D) colored by p-value, where terms containing more tend to have a more significant p-value in RRGs. (E) demonstrated GO terms and KEGG pathways enrichment of RRGs.

Figure 3

Prognostic value of RRGs and the development of a prognostic index based on RRGs (A) Risk ratio forest plot showed the prognostic value of the gene; (B) Distribution of prognostic index and survival status of patients in different groups and heat map of the expression profile of the included RRGs.(C) The correlation of genes. Red represents the positive correlation, while the blue represents the negative correlation. (D) Patients in the high-risk group have a shorter overall survival. (E)Survival-dependent receiver operating characteristic (ROC) curves validate the prognostic significance of RRGs-based prognostic indicators

Figure 4

(A,B) multivariate Cox regression analysis in Ewing's sarcoma. (C) The expression of prognostic-related RRGs in high-risk group and low-risk group respectively. (D,E) Construction of the nomogram model. (D) Calibration chart of Nomogram that predicts the probability of OS occurring in 1, 3 and 5 years. (E) The Nomogram model predicts the 1-year, 3-year and 5-year OS probability of Ewing's sarcoma occurrence. ROC curve and the AUC values of one-year, three-year and five-year survival rates are shown in the figure.

Figure 5

Relations between immune cells and prognostic genes (APOBEC2, CAPRIN1, CPEB3, EE1A2, INTS6, MYEF2, NXT2, PAN3, PAPD4, PRPF38B).

Figure 6

Relations between immune cells and prognostic genes (APOBEC2, CAPRIN1, CPEB3, EEF1A2, INTS6, MYEF2, NXT2, PAN3, PAPD4, PRPF38B).